IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lustig et al.

Serial No. ~~08/975,614~~ 09/163,913

Filed: November 21, 1997

For: *Nuclear Hormone Receptor Drug Screens*

Group Art Unit: 1646

Examiner: Pak, M.

Attorney Docket No. T97-012

NOP 12/11/02

DECLARATION UNDER RULE 132

I, Keith R. Yamamoto, declare and state as follows:

1. I am a Professor of Biochemistry in the Department of Biochemistry and Biophysics at the University of California, San Francisco (UCSF) , and also serve as Director of the Biochemistry and Molecular Biology Program, and Chairman of the Department of Cellular and Molecular Pharmacology at UCSF. I have been a member of the American Academy of Arts and Sciences since 1989 and a member of the National Academy of Sciences since 1990. Over decades of research in cell biology, I have been presented numerous nationally recognized honors and awards, have served and continue to serve on numerous academic editorial boards and numerous federal government public advisory committees. I am a recognized expert in the field of cell biology and have authored hundreds of publications in this field. I serve as member of the scientific advisory board of Tularik, Inc., the assignee of this application.

2. Heery et al. (1997, Nature 387,733-36) describes three experiments: the first is an in vivo yeast-based two-hybrid experiment wherein a DNA-binding domain fusion protein comprising LXXLL motifs activated transcription through a ligand-binding domain of an estrogen receptor (Fig.1). The second experiment is a GST pull-down experiment wherein GST-ER fusion proteins pulled down in vitro translated ^{35}S-labeled natural-sequence SRC-1 proteins, but not otherwise identical mutant-sequence SRC-1 proteins wherein all four functional LXXLL motifs were disabled (Fig.3a). In this experiment, the natural sequence SRC-1 pull down was inhibited by uM concentrations of LXXLL peptides (Fig.3b). The third experiment showed that natural SRC-1 but not mutant SRC-1 increased activation of estrogen receptor in HeLa cells transiently transfected with a reporter plasmid (Fig.3c).

One skilled in the art would not construe Heery to suggest the feasibility of assaying direct, in vitro LXXLL peptide binding to purified receptor; in fact, to one skilled in the art, Heery suggests the opposite - that such an assay would not be feasible. First, Heery provides no data suggesting an LXXLL peptide can directly bind the receptor. Heery=s two-hybrid transcriptional activation is performed within yeast cells, the GST-pull down assay is performed in a crude cellular lysate, and the third, transient transfection experiment is cell-based. One skilled in the art would recognize that all of these experiments report both higher and lower order complex formation and that none of them implies

direct peptide-receptor binding.

Similarly, none of Heery=s data imply that an LXXLL peptide is sufficient to bind the receptor; in fact, they suggest the opposite. For example, one skilled in the art would recognize that Heery does not report any GST-pull down of an LXXLL peptide, nor any data wherein a single LXXLL motif was disrupted, but only wherein all four were disrupted. In fact, one skilled in the art would conclude that the author=s failure to provide such data suggests that single LXXLL-motif disruptions may not have worked. In fact, this negative inference is further compelled by the subsequent inhibition experiments, wherein the authors only report data wherein uM concentrations of peptide were required to inhibit pull down - orders of magnitude higher than the amount of the SRC-1 protein present (the disclosed in vitro transcriptional yield is on the order of a few nM). One skilled in the art would conclude that the disproportionately high concentration of peptide necessary to inhibit SRC-1 pull down suggests that LXXLL peptides do not provide sufficient receptor binding affinity to permit a direct, binding assay.

In short, none of Heery=s data suggest that the single peptides would be able to directly bind purified receptor proteins. Viewed through the eyes of one skilled in the art, Heery teaches away from an assay that relies on direct, in vitro, ligand-dependent LXXLL peptide binding to purified receptor, particularly one that requires lower, particularly sub-micromolar peptide concentrations.

3. In fact, when the project that led to this invention was first proposed over five years ago, neither I nor Steven McKnight, serving as scientific advisors, expected the ligand-dependent NHR binding assay to work with small peptide sensors as claimed. At the time, Tularik Inc. was seeking to set up a commercial assay for NHR ligands and we felt that existing assay formats, such as the gel-based coactivator dependent receptor ligand assay (Krey et al., 1997, Mol Endocrinol 11, 779-791), were not suitable for high-throughput applications. Dr. McKnight and I were well aware of publications, including the cited Heery et al., which had attempted to characterize NHR - coactivator binding requirements, and had identified coactivator regions necessary for binding. After carefully reviewing these publications, we concluded that the evidence provided by these papers did not suggest that small single LXXLL-motif peptides would be sufficient to bind receptor in a ligand-dependent manner sufficient to construct a binding assay as claimed. In fact, as noted above, we inferred the opposite B that Heery=s failure to provide any direct binding evidence and their disclosure that orders of magnitute higher concentrations of peptides were necessary to inhibit coactivator pull-down, suggest to us skilled in the art that such small peptides did not, and would not, provide sufficient binding specificity and affinity to construct a direct binding assay as claimed. Hence, at the project review meeting, both Dr. McKnight and I communicated our pessimistic assessment of the likelihood of succeeding with the proposed assay development project.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: August 29, 2002

Keith R. Yamamoto

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lustig et al.

Serial No. 08/975,614

Filed: November 21, 1997

For: *Nuclear Hormone Receptor Drug Screens*

Group Art Unit: 1646

Examiner: Pak, M.

Attorney Docket No. T97-012

DECLARATION UNDER RULE 132

I, Jin-Long Chen, declare and state as follows:

1. I am a Director of Biology at Tularik Inc, the assignee of the above application. I am an expert in the field of cell biology, with particular expertise in nuclear hormone receptor biology. I am a coinventor of the above application. I hold a Ph.D. in Molecular Biology from the University of California at Berkeley.

2. Heery et al. (1997, Nature 387,733-36) describes three experiments: the first is an in vivo yeast-based two-hybrid experiment wherein a DNA-binding domain fusion protein comprising LXXLL motifs activated transcription through a ligand-binding domain of an estrogen receptor (Fig.1). The second experiment is a GST pull-down experiment wherein GST-ER fusion proteins pulled down in vitro translated ^{35}S-labeled natural-sequence SRC-1 proteins, but not otherwise identical mutant-sequence SRC-1 proteins wherein all four functional LXXLL motifs were disabled (Fig.3a). In this experiment, the natural sequence SRC-1 pull down was inhibited by uM concentrations of LXXLL peptides (Fig.3b). The third experiment showed that natural SRC-1 but not mutant SRC-1 increased activation of estrogen receptor in HeLa cells transiently transfected with a reporter plasmid (Fig.3c).

One skilled in the art would not construe Heery to suggest the feasibility of assaying direct, in vitro LXXLL peptide binding to purified receptor; in fact, to one skilled in the art, Heery suggests the opposite - that such an assay would not be feasible. First, Heery provides no data suggesting an LXXLL peptide can directly bind the receptor. Heery=s two-hybrid transcriptional activation is performed within yeast cells, the GST-pull down assay is performed in a crude cellular lysate, and the third, transient transfection experiment is cell-based. One skilled in the art would recognize that all of these experiments report both higher and lower order complex formation and that none of them implies direct peptide-receptor binding.

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receptor; in fact, they suggest the opposite. For example, one skilled in the art would recognize that Heery does not report any GST-pull down of an LXXLL peptide, nor any data wherein a single LXXLL motif was disrupted, but only wherein all four were disrupted. In fact, one skilled in the art would conclude that the author=s failure to provide such data suggests that single LXXLL-motif disruptions may not have worked. In fact, this negative inference is further compelled by the subsequent inhibition experiments, wherein the authors only report data wherein uM concentrations of peptide were required to inhibit pull down - orders of magnitude higher than the amount of the SRC-1 protein present (the disclosed in vitro transcriptional yield is on the order of a few nM). One skilled in the art would conclude that the disproportionately high concentration of peptide necessary to inhibit SRC-1 pull down suggests that LXXLL peptides do not provide sufficient receptor binding affinity to permit a direct, binding assay.

In fact, our own data showed that there is no necessary correlation between inhibitory activity and receptor binding. For example, SRC 1427-1440 is reportedly inhibitory in Heery=s coactivator - NHR binding inhibition assay (Fig.3a), yet we found the exact same peptide insufficient to directly bind three different receptors in our binding assay (Specification, p.6, lines 24-27).

In short, none of Heery=s data suggest that the single peptides would be able to directly bind purified receptor proteins. Viewed through the eyes of one skilled in the art, Heery teaches away from an assay that relies on direct, in vitro, ligand-dependent LXXLL peptide binding to purified receptor, particularly one that requires lower, particularly sub-micromolar peptide concentrations.

3. In fact, when the project that led to this invention was first proposed over five years ago, neither I nor Professors Keith Yamamoto and Steven McKnight, expected the ligand-dependent NHR binding assay to work with small peptide sensors as claimed. At the time, Tularik Inc. was seeking to set up a commercial assay for NHR ligands and we felt that existing assay formats, such as the gel-based coactivator dependent receptor ligand assay (Krey et al., 1997, Mol Endocrinol 11, 779-791), were not suitable for high-throughput applications. Drs. McKnight, Yamamoto and I were well aware of publications, including the cited Heery et al., which had attempted to characterize NHR - coactivator binding requirements, and had identified coactivator regions necessary for binding. After carefully reviewing these publications, we concluded that the evidence provided by these papers did not suggest that small single LXXLL-motif peptides would be sufficient to bind receptor in a ligand-dependent manner sufficient to construct a binding assay as claimed. In fact, as noted above, we inferred the opposite B that Heery=s failure to provide any direct binding evidence and their disclosure that orders of magnitute higher concentrations of peptides were necessary to inhibit coactivator pull-down, suggest to us skilled in the art that such small peptides did not, and would not, provide sufficient binding specificity and affinity to construct a direct binding assay as claimed. Hence, at the project review meeting, Drs. McKnight, Yamamoto and I communicated our pessimistic assessment of the likelihood of succeeding with the proposed assay development project.

4. Our patent application teaches that L_1-L_3 are independently selected from hydrophobic amino

acids, preferably leucine or isoleucine, more preferably leucine. See Specification, p.4, lines 26-28. The claimed methods require a peptide which provides direct, in vitro ligand-dependent binding to a nuclear hormone receptor. The Specification exemplifies the sensors and methods with a wide variety of suitable exemplary peptides with several receptors. Specification, p.5, line 23 - p.7, line 11. Though our exemplified list focused on our preferred embodiment wherein L_1- L_3 are leucine, we have in fact successfully practiced the claimed assay wherein L_1- L_3 are non-leucine hydrophobic amino acids, such as isoleucine and valine (see attachment e.g. TUK-1620-20, 1621-23, 1620-23).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: August 29, 2002

Jin-Long Chen

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα 25ng/uL 24,25 ex, 8uM ΔmP	LXRβ, 25ng/uL 24,25 ex, 8uM ΔmP	FXR, 35ng/uL cDCA, 80uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.5uM ΔmP	PPARγ, 25ng/uL GW, 0.1uM ΔmP
SRC-1 632-640									
TUK-1385-60	R-	KLVQLLTTT	37	57.95	15	2.45	87.1	20.45	23.15
TUK-1385-66	R-	KLVQLLTTT	9.95	8.85	6.95	10.0	65.95	8.05	15.6
TUK-1387-60	R-G-	KLVQLLTTT	23.8	39.35	13.45	2.15	141.1	26.6	3.05
TUK-1387-62	R-G-	KLVQLLTTT	28.05	14.3	1.6	75.85	185.4	13.75	24.75
TUK-1387-70	R-G-	KLVQLLTTT	14.95	23.75	19.8	8	152.3	33.3	7.75
TUK-1387-72	R-G-	KLVQLLTTT	2.9	26	8.3	10.8	154.8	11.4	6.95
SRC-1 689-696									
TUK-1668-31	R-	ILHRLL	14.75	13.75	5.5	5.8	-3.65	12.55	6.95
TUK-1668-36	R-	ILHRLL	16.05	0.55	16.95	4.4	-0.95	12.4	6.55
SRC-1 689-696									
TUK-1371-58	R-	ILHRLLQE	70.25	157.7	41.25	84.4	108.7	41.5	90.6
TUK-1371-62	R-	ILHRLLQE	19.5	21	107.7	77.5	56	23.15	-2.05
TUK-1373-57	R-G-	ILHRLLQE	34.45	42.7	16.9	32.5	79.95	23.1	23
TUK-1373-63	R-G-	ILHRLLQE	27.05	70.35	12.5	13.75	98.6	17.05	0.75
SRC-1 746-756									
TUK-1474-52	R-	EQLLRYLLDKD	33.2	46.85	16.7	12.8	93.2	13.15	4.6
TUK-1474-61	R-	EQLLRYLLDKD	30.75	36.3	23.65	8.85	66.7	9.4	-1.7
SRC-1 747-756									
TUK-1473-70	R-	QLLRYLLDKD	27.35	133.05	101.55	34.45	101	37.15	25.5
TUK-1473-75	R-	QLLRYLLDKD	33.75	129.3	93.15	5.65	87.55	17.45	13.4
SRC-1 744-753									
TUK-1457-46	R-	LLRYLL	20.0	-35.4	0.15	-9.25	-8.05	-0.3	-3.95
TUK-1457-49	R-	LLRYLL	3.45	-37.65	-6.1	1.25	-1.2	11.55	-6.05
TUK-1670-44	R-	LLRYLL	19.55	-6.2	8.75	5.60	-0.35	3.9	0.6
TUK-1670-46	R-	LLRYLL	6.39	-14.15	-3	16.95	0.75	5.05	1.7
SRC-1 749-754									
TUK-1459-18	R-	LLRYLLD	14.35	23.2	37.05	0.2	33.55	5.5	-5.65
TUK-1453-22	R-	LLRYLLD	17.95	-5.45	-17.7	6.35	33	5.2	3.0
SRC-1 749-759									
TUK-1391-73	R-	LLRYLLDK	309.9	75.3	99.65	72.65	37.9	43.85	37.7
TUK-1391-76	R-	LLRYLLDK	117.35	7.2	22.15	83.05	45	37.65	12.65
TUK-1393-58	R-G-	LLRYLLDK	42.75	69	20.5	5.1	69.8	21.15	11
TUK-1393-61	R-G-	LLRYLLDK	38.8	51.15	22.35	3.2	45.1	13	13
SRC-1 748-756									
TUK-1472-61	R-	LLRYLLDKD	133.05	75.95	107.7	6.25	77.15	46.5	35
TUK-1472-64	R-	LLRYLLDKD	53.4	51.75	99.1	33.15	64.9	30.7	25.35
SRC-1 749-753									
TUK-1459-43	R-	LRYLL	9.55	17.4	7.05	73.25	8.1	18.2	8.55
TUK-1459-46	R-	LRYLL	14.45	5.35	3.5	3.75	-1.8	20.9	4.4
SRC-1 749-754									
TUK-1455-40	R-	LRYLLD	0.3	74.15	57.5	0.5	11.25	27.4	37.1
TUK-1455-43	R-	LRYLLD	8.45	11.1	3.65	2.5	4.3	10.6	4.25
SRC-1 1427-1440									
TUK-1389-71	R-G-	PQAQQKSLLQQLLT	5.7	16.85	11.2	6.7	10.85	10.1	10.15
SRC-1 1434-1441									
TUK-1381-67	R-	LLQQLLTE	7.95	27.1	9	17.7	42.7	10.1	34.15
TUK-1381-69	R-	LLQQLLTE	38.8	83.1	47	69.4	48.6	31.65	27.65
TUK-1383-55	R-G-	LLQQLLTE	1.95	51.15	22.15	75.35	52	4	6.05
TUK-1383-61	R-G-	LLQQLLTE	15.4	50.3	10.95	6.9	66.4	13.65	6.05
RIP-140 132-139									
TUK-1631-27	R-	LLASLLQS	59.35	62.35	35.5	7.1	100.75	64.25	140.9
TUK-1631-29	R-	LLASLLQS	17.35	48.15	15.35	3.85	59.05	51.5	33.45
TUK-1631-30	R-	LLASLLQS	21.8	31.85	10.95	3.1	37.35	30.55	36.75
RIP-140 184-191									
TUK-1616-31	R-	HLKTLLKK	36.15	4.35	3.15	19.35	25.3	30.7	17.95
TUK-1616-35	R-	HLKTLLKK	2	9.35	3.2	-2.25	14.05	34.1	19.9
RIP-140 366-373									
TUK-1632-38	R-	QLALLLSS	9.05	16.5	12.5	10.1	19.4	17.35	18.35
RIP-140 379-386									

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM	LXRα, 35ng/uL 24,25 ex, 30uM	LXRβ, 25ng/uL 24,25 ex, 30uM	FXR, 60ng/uL cDCA, 30uM	ERβ, 15ng/uL b-Estr., 0.1uM	PPARγ, 30ng/uL BRL, 0.2uM	PPARγ, 65ng/uL GW, 0.1uM
			ΔmP	ΔmP	ΔmP	ΔmP	ΔmP	ΔmP	ΔmP
TUK-1693-31	R-	LLLFLLKS	114.2	135.35	90.3	13.2	141.5	84	94.2
TUK-1693-32	R-	LLLHLLKS	41.55	46.25	11.3	84.95	85.55	22.5	37.3
RIP-140 498-506									
TUK-1375-47	S-	VTLLQLLLG	31.05	69.2	38.85	13.95	72.25	1.2	113.35
TUK-1375-51	R-	VTLLQLLLG	44.9	74.45	-1.55	6.7	41.5	1.1	105
TUK-1377-72	R-G-	VTLLQLLLG	38	58.45	3.15	17.7	67.5	7.35	9.75
TUK-1433-69			26.65	60.55	23	11.15	85.95	35.1	50.55
RIP-140 712-719									
TUK-1618-63	R-	VLQLLLGN	12.5	45.6	-1.3	16.95	9.55	13	7.6
TUK-1618-67	R-	VLQLLLGN	11.05	20.1	10.45	7.6	17.95	15.05	10.35
RIP-140 818-825									
TUK-1644-48	R-	LLSRLLRQ	99.75	43.1	33.35	89.15	32.9	16.85	10.4
TUK-1644-49	R-	LDSRLLRQ	30.9	8.7	7.8	13.85	38.8	10.45	13.95
RIP-140 935-942									
TUK-1608-43	R-	VLKQLLLS	38.05	18.15	11.3	9.8	55.6	30.1	18.65
RIP140									
TUK-1614-43	R-	YLEQLLMN	8.75	6.25	7.6	2.35	10.45	8.85	-5.65
CBP 68-									
TUK-1613-24	R-	QLSQLLRG	8.7	13.1	2.95	-1.35	7.6	18.05	10.95
TUK-1615-26	R-	QLSQLLRG	2.45	39.75	7.8	10	13.3	17.1	3.95
TUK-1615-27	R-	QLSQLLRG	8.55	5.9	4.6	-0.25	6	12.85	10.65
CBP 356-									
TUK-1637-30	R-	QLVLLLSA	10.6	27.6	17.1	9	19.6	11.3	11.95
TUK-1637-42	R-	QLVLLLSA	3.65	13.15	-8.1	10.35	17.15	21	2.35
p120 239-246									
TUK-1628-37	R-	LLSELLKK	44	54.5	15.25	78.5	37.55	6.1	15.75
TUK-1628-39	R-	LDSELLKK	16.2	18.6	-3.5	95.55	24.8	7.7	12.15
p120 308-315									
TUK-1629-33	R-	TLSRLLEA	17.8	74.1	24.95	68.25	36.1	15.1	7.1
TUK-1629-36	R-	TLSRLLEA	4.85	31.1	4.25	77.3	15.45	13.35	-2.2
TRIP2 23-									
TUK-1642-36	R-	MLMNLLED	36.05	73	34.4	0.6	51.95	32.65	30.1
TUK-1642-39	R-	MLMNLLED	11.5	42.55	32.65	8.15	37.5	17.1	8.5
TRIP4 36-									
TUK-1643-35	R-	RLAVLLPG	11.75	0.05	3	10.7	4.6	9.2	-2.05
TUK-1643-37	R-	RLAVLLPG	2.4	9.55	-1.95	6.15	-1.2	18.65	8.05
TRIP5 26-									
TUK-1640-40	R-	ELSDLLEV	15.95	51.95	9.15	34.9	30.1	12.15	12.35
TUK-1645-42	R-	ELHDLLEV	8.7	14.95	5.1	80.15	14.75	30.35	12.55
TRIP8 36-									
TUK-1641-39	R-	TLHDLLTT	6.9	13.35	12.25	6.3	32.8	21.1	11.5
TRIP9 73-									
TUK-1634-55	R-	FLDFLLGF	0.7	36.8	-17.3	8.3	-13.05	1.2	-1.85
TUK-1634-57	R-	FLDFLLGV	-36.95	-45.3	-59.4	-2	-33.75	-16.4	-21.05
TRIP9 356-									
TUK-1636-34	R-	VLELLLRA	19.15	24.75	13.15	10.35	31.65	17.05	9.9
TUK-1636-59	R-	VLELLLRA	9.1	24.05	-4.75	-0.8	16.5	7.9	1.5
TRIP9 298-									
TUK-1639-42	R-	YLARLLRA	83.45	11.9	6.9	78.65	75.6	10.05	3.45
TIF1 722-									
TUK-1638-48	R-	ILTSLLLN	108.6	75.3	15.75	10.1	137.25	17.65	31.9
TUK-1638-50	R-	ILTSLLLN	38.6	11.15	2.35	5.15	89.2	11.4	4.35
TIF2 640-									
TUK-1635-36	R-	KLLQLLTT	55.05	80.85	40.9	7.05	144.1	99.6	33.55
TUK-1635-39	R-	KLLQLLTT	13.5	37.2	11	13.3	80.95	20.7	24.9
TUK-1635-40	R-	KLLQLLTT	9.15	30.25	7.25	13.15	87.6	7.85	13.55

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα, 25ng/uL 24,25 ex, 50uM ΔmP	LXRβ, 20ng/uL 24,25 cx, 3uM ΔmP	FXR, 25ng/uL cDCA, 50uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.6uM ΔmP	PPARδ, 25ng/uL GW, 0.1uM ΔmP
TIF3 37-					26.45	77.6	91.3	56.95	62.25
TUK-1640-37	R-	TLRSLLLD	118.3	72.15	19.05	93.65	95.25	45.75	64.05
TUK-1640-40	R-	TLRSLLLN	116.2	61	19.35	4.25	35.7	32.55	14.7
TUK-1640-41	R-	TLRSLLLW	34.85	11.9					
Non-co-activator									
TUK-1557-43	R-	ILRRLLQE	128.85	132.5	20.85	10.9	92.7	36.75	32.4
TUK-1557-52	R-	ILRRLLQE	69.25	50.05	18.7	8.55	91.25	37.65	61.45
TUK-1558-60	R-	ILRRLLQE	186.75	72.4	18.45	12.55	108.85	29.5	32.4
TUK-1559-53	R-	ILRRLLQE	147.85	97	36.15	7.6	96.05	33.35	55.75
TUK-1559-58	R-	ILRRLLQE	73.25	66.05	44.8	5.2	145.9	36.6	36.7
TUK-1560-21	R-	ILRRLLQE	132.25	81.3	37.15	71.3	106.7	26.65	30.65
TUK-1560-36	R-	ILRRLLQE	232.6	142.9	33.25	77.4	149.8	105.8	130.4
TUK-1560-52	R-	ILRRLLQE	255.6	142.6	86.95	80.65	161.9	107.7	146.2
TUK-1560-55	R-	ILRRLLQE	103.35	73.9	20.15	6.65	91.35	26.7	31
TUK-1562-37	R-	ILHRLYQE	21.35	45.55	17.3	3.35	32.3	21.85	14.95
TUK-1562-39	R-	ILHRLYQE	9.55	7.35	1.1	7.25	14.85	9.2	0.3
TUK-1563-37	R-	IIHRLLQE	28.2	62.75	16.1	12.3	66.15	17.85	16.75
TUK-1563-39	R-	ILHRIIQE	28.05	7.55	11.9	12.45	23.15	8.1	6.75
TUK-1564-38	R-	IIHRLLQE	30.2	81.75	10.55	15.9	67.95	21.7	20.85
TUK-1564-40	R-	IIHRLLQE	13.15	10.15	-6.4	5.55	17.85	5.2	18.9
TUK-1565-40	R-	ILHRLFQE	9.55	10.95	1.45	7.8	11.35	14.35	8.4
TUK-1565-42	R-	ILHRLFQE	6.7	3.9	13.55	11.45	6.9	9.19	2.55
TUK-1568-39	R-	ILHRFLQE	14.25	39.5	10.3	3.95	30.75	9.55	11.7
TUK-1568-41	R-	YLHRFLQE	15	10.95	2.65	10.4	21.3	13.75	7.55
TUK-1569-37	R-	IFHRLLQE	13.85	5.6	17.25	4.65	9.35	6.75	11.65
TUK-1569-39	R-	IFHRLLQE	14.6	11.0	-1.1	4.85	3.55	13.1	4.95
TUK-1570-39	R-	FLHRLLQE	53.85	134.95	43.6	12.85	60.6	23.05	34
TUK-1570-41	R-	FLHRLLQE	19.4	24.55	7.65	1.3	21.05	21.25	9.7
TUK-1571-40	R-	ILRYLLQE	132.65	111.6	30.4	84.15	152.7	45.5	55.45
TUK-1571-50	R-	ILRYLLQE	95.85	53.05	19.1	75.85	61.5	14.35	17.8
TUK-1575-33	R-	LLHRLLDK	-0.6	134.15	112.6	10.4	42.7	32.8	26.3
TUK-1575-37	R-	LLHRLLDK	17.5	46.6	30.95	10.3	25.35	16.65	11.25
TUK-1576-45	R-	ILHRLLNE	55	135.65	50.55	16.95	87.35	15.25	26.05
TUK-1576-47	R-	ILHRLLNE	14.35	28.65	12	30.6	32.4	7.8	6.75
TUK-1577-43	R-	ILHRLLQD	74.9	132.45	51.45	12.65	102.4	36.95	39.5
TUK-1577-45	R-	ILHRLLQD	24.55	29.8	35.65	80.05	51.7	22.8	-0.8
TUK-1578-38	R-	ILHRLLND	43.7	148.9	52.65	30.7	73.7	28.55	34.45
TUK-1578-41	R-	ILHRLLND	23.75	32.8	17.45	14.9	20.05	14.4	3.65
TUK-1579-39	R-	ILHRLLED	30.1	33.3	3.7	19.15	48.35	18.35	10.2
TUK-1580-41	R-	ILHRLLEE	25.25	82.15	91.55	3.45	56.6	7.85	14.65
TUK-1580-42	R-	ILHRLLEE	21.25	36.4	12.75	17.15	37.2	10.4	2.9
TUK-1581-43	R-	ILHRLLYE	34.95	20.1	9.65	7.95	65.55	30.45	9.05
TUK-1583-39	R-	ILHRLLKE	34.3	16.7	-0.85	11.5	54.3	27.9	5.95
TUK-1586-49	R-	ILHRLLEK	32.3	46.45	51.55	7.75	22.25	11.75	-0.9
TUK-1586-50	R-	ILHRLLEK	16.09	49.4	11.1	13.9	31.1	21.6	2.35
TUK-1587-40	R-	ILHRLLYE	48.4	38.45	39.8	7.4	66.45	23.75	28.09
TUK-1587-41	R-	ILHRLLIE	37.75	23.3	30.5	9.15	57.75	30.15	10.85
TUK-1588-45	R-	ILHRLLEI	40.9	62.2	50.35	12.25	30.3	16.15	4.5
TUK-1588-48	R-	ILHRLLEI	-8.16	21.5	-1.35	52.4	47.25	30	12.85
TUK-1589-40	R-	ILHRLLVE	46.5	89.1	31.05	76.25	62.1	14.55	17.95
TUK-1589-42	R-	ILHRLLVE	12.95	19	9.7	11.35	23.2	10.35	8.5
TUK-1594-43	R-	YLHRLLEY	48.35	78.15	35.1	7.05	41.35	7.55	13.8
TUK-1594-44	R-	ILHRLLEY	14.85	16.1	10.75	10.35	26.2	12.0	12.25
TUK-1595-40	R-	ILQRYLQE	26.05	17.75	1.2	5.1	18.2	9.55	15.8
TUK-1597-38	R-	ILQRSLLQS	17.65	5.85	4.25	-8.4	9.3	13.1	4.4
TUK-1599-47	R-	ILCIRLLQE	100.9	44	23.45	9.05	81.55	23.4	24.25
TUK-1601-46	R-	ILRCIRLLQE	188	150.8	51.45	19.3	109.35	75.1	87.8
TUK-1601-49	R-	ILDCIRLLQE	96.45	44.8	23.6	3.85	107.7	34.7	39.15
TUK-1603-51	R-	ILROLLQE	167.15	78.3	44.3	8.4	102.8	47.75	47.5
TUK-1606-44	R-	ILDRRLLQE	133.6	56.3	24.7	76.45	103.35	31.5	30.05
TUK-1607-43	R-	FLRKLLQE	85	40.55	21.6	13	48.5	30.75	36.95
TUK-1607-45	R-	FLRKLLQE	89	40.55	11.6	13	48.5	20.75	26.95
TUK-1610-42	R-	ILRELQLQE	56.75	35.35	6.8	7.75	30.5	11.75	-3.75
TUK-1611-36	R-	ILRKQLLQE	230.85	136.35	116.1	76.05	155.4	110.4	188.5
TUK-1611-38	R-	ILRRMLLQE	146.3	38.25	33.45	64.95	95.95	37.45	47.85
TUK-1612-40	R-	IQLKRLLQE	90.65	69.35	19.35	3.9	51.05	8.35	15.4
TUK-1612-42	R-	IDLKRLLQE	84.95	42.85	11.7	0.15	80	20.45	15.5
TUK-1613-39	R-	DLLRKLLQE	152.75	46.65	18.35	9.7	68.5	31.0	37.7
TUK-1620-20	R-	ILRKAVLQE	131.45	77.55	34.25	5.45	142.7	63.9	70.35
TUK-1620-23	R-	ILRKAVLQE	130.7	50.95	8.15	7.15	84.7	21.7	24.6
TUK-1621-23	R-	IDVRKLLQE	234.4	61.85	16	5.8	105.15	18.9	31.8
TUK-1621-25	R-	IDVRKLLQE	130	29.85	-3.85	9.3	56.9	38.65	7
TUK-1622-31	R-	DVLRKLLQE	187.65	152.15	54.35	10.25	153.7	80.15	166.8
TUK-1622-29	R-	DVLRKLLQE	68.8	59.65	23.15	16.3	69.05	63.3	30.2
TUK-1624-21	R-	IDLRKLLLQE	181.65	103	56.95	11.65	153.6	60.6	55.45

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα 18ng/uL 24,25 ex, 3uM ΔmP	LXRβ 22ng/uL 24,25 ex, 3uM ΔmP	FXR, 20ng/uL CDCA, 80uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ 25ng/uL BRL, 0.6uM ΔmP	PPARγ 30ng/uL GW, 0.1uM ΔmP
TUK-1624-22	R-	IaLSKLLLQE	132.65	69.05	32.45	12.8	87.45	28.3	17.55
TUK-1624-23	R-	IaLRKLLQE	132.1	64.4	14.7	1.15	73.4	17.25	0.6
TUK-1625-21	R-	ILRciLLQE	274.6	88.25	40.5	11.4	109.75	91.9	113.4
TUK-1625-23	R-	ILRciLLQE	219.65	79.8	27.8	6.6	91.45	17	26.9
TUK-1626-45	R-	ILRKciLLLQE	279.4	139.6	37	27.2	144.8	100.55	143.25
TUK-1626-48	R-	ILRKciLLLQE	176.65	67.5	12.45	1.75	93.8	30.8	37.95
TUK-1627-45	R-	ILRciRciLLQE	139.2	89.8	48	10.95	141.2	67.3	73.55
TUK-1627-46	R-	ILRciRciLLQE	58.05	23.95	7.15	7.6	110	18	31.7
TUK-1630-19	R-	ILRKLLQD	183.2	84.9	95.75	14.35	106.85	79	113.3
TUK-1630-22	R-	ILRKLLQE	111.9	63.6	30.75	6.95	88.4	24.05	33.55
TUK-1646-21	R-	ILRKLLQEi	141.15	56.25	35.45	14.35	75.65	60.05	65.3
TUK-1646-24	R-	ILRKLLQci	160.75	52.05	34.2	90.6	66.75	45.05	29.4
TUK-1647-22	R-	ILRKLLQEci	249.3	76.3	35.95	79.9	85.15	88.85	83.75
TUK-1647-25	R-	ILRKLLQEci	129.1	25.8	4.63	17.7	46.3	28.2	25.65
TUK-1648-27	R-	ILRKLLciE	246.5	131	117.6	26.4	107.3	123.9	157.2
TUK-1648-30	R-	ILRKLLciE	122.2	60.7	61.2	12.7	100.3	37.95	95.35
TUK-1649-29	R-	ILRKLLEciE	165.9	90.25	43.45	76.65	105.6	58.5	51
TUK-1649-30	R-	ILRKLLEciE	137.4	93.8	35.5	77.9	100.25	40.5	42.05
TUK-1650-29	R-	ILRKLLEciEi	104.55	35.3	4.85	5.9	63.5	28.45	28.05
TUK-1651-36	R-	ILRKLciRci	252.5	75.15	56.65	37.4	98.05	89.3	137.75
TUK-1651-39	R-	ILRKLciRci	163.35	43.6	20.55	10.45	66.55	29.5	29.7
TUK-1652-35	R-	ILRKLEciEi	186.4	73.3	45.7	12.45	83.9	81.85	134.9
TUK-1652-37	R-	ILRKLciEi	131.55	64.25	16.7	76.3	63.4	19.8	26.9
TUK-1653-35	R-	ILRKLLSciRci	155.76	60.65	51.95	81.95	38	80.35	79.4
TUK-1653-37	R-	ILRKLLSciRci	126.3	36.1	14.85	16.3	54	95.6	45.4
TUK-1654-27	R-	ILRKLaLQE	52.35	72.3	36.75	21.7	47.95	18.45	94.15
TUK-1654-29	R-	ILRKLaLQE	17.05	23.05	-15.8	13.65	20.75	8.35	17.95
TUK-1655-37	R-	ILRKLaYQE	97.75	85.0	111.7	81.95	107	119.45	156.3
TUK-1655-38	R-	ILRKLaYQE	52.65	84.9	54.9	8.3	51.7	85.05	83.2
TUK-1656-37	R-	IaLRKLLQE	81.9	69.2	49.65	5.3	63.75	31.6	28.1
TUK-1656-30	R-	IaLRKLLQE	56.8	54.75	47.9	13.8	78.1	54.0	43.95
TUK-1657-36	R-	bILRKLLQE	162.3	66.95	33.7	78.9	129.3	103.25	153.2
TUK-1657-38	R-	bILRKLLQE	172.78	50.9	2.4	9.5	78	37.8	37.5
TUK-1750-48	R-	ILRKLLQEEbiot	176.2	47.15	34	11.7	97.3	42.6	62.35
TUK-1829-39	R-	ILRKLLQGbiot	40.4	56.55	84.9	17.7	59.3	26.65	17.2
TUK-1829-50	R-	ILRKLLEGbiot	25.33	31.95	14.85	13.45	30.65	31.2	17.75
TUK-1831-63	R-	ILRKLLQEbiot	31.35	29.5	2.6	4.1	37.7	14.7	19.3
TUK-1833-56	R-	ILRKLLEEbiot	32.33	83.85	61.95	12.15	53.45	15.75	-0.6
TUK-1833-58	R-	ILRKLLEEbiot	25.45	59.15	13.35	11.53	25.35	13.95	31.35
TUK-1835-39	R-	LLRKLLEGbiot	19.55	39.2	20.9	-3.25	67.9	33.8	28.6
TUK-1837-36	R-	LLLRLLEGbiot	28.75	56.2	23.65	19.3	93.6	32.7	31.45
TUK-1839-39	R-	ILRSLLLEbiot	94.2	47.7	18.5	6.05	34.1	31.75	38.3
TUK-1841-38	R-	ILRKLLKEbiot	19.25	49.55	21.9	80.95	45.05	10.3	10.25
TUK-1843-40	R-	ILRciKLLQEbiot	75.7	57.75	8.8	3.75	147.4	39.85	34.05
TUK-1849-48	R-	ILRK-morlinoLQEbiot	198.95	59.8	33	16.1	91.9	35.6	39.9
TUK-1851-47	R-	ILRKL-morlinoQEbiot	63.4	38	21.5	13.15	31.2	6.1	4.45
STAT									
TUK-1617-45	R-	CACTVPVQDLYC	111.3	53.35	24.85	19.8	65.75	48	51.2
TUK-1617-48	R-	CAGTVPVQDLYC	38.8	-17.2	-7.95	6.8	5.6	6.1	10.45
YAMAMOTO									
TUK-1675-27		R-KHKILHRLLQDSGSPVDLAK	19.75	18	17.3	-5.1	34.4	13.75	12.85
TUK-1675-29		R-KHKILHRLLQDSGSPVDLAK	20.75	24.45	10.3	8.6	40.65	20.65	11.25
TUK-1679-54	R-	KHKILHRFLQDSSS	5	1.6	6.75	3.4	8.2	9.05	11.45
TUK-1679-59	R-	KHKILHRFLQDSSS	-1.7	10	8.55	10.4	0.3	19.25	-3.9
TUK-1681-34	R-	KHKILHRLFQDSSS	0.1	7.25	4.75	-1.65	0.35	2.8	10.25
TUK-1683-32	R-	KHKILHRLLQDSSS	12.35	2.95	11.75	8.65	26.45	21.05	5.95
TUK-1685-31	R-	KHKAAERLLQDSSS	1.65	-22.55	-3.35	6.08	2.15	14.85	11.4
TUK-1685-34	R-	KHKAAHRLLQDSSS	11.85	4.65	1.5	5.5	5.9	20.3	14.05
TUK-1689-40	R-	KHKILAALLQDSSS	15.85	30.45	0.9	3.65	27	16.2	15.95
TUK-1689-42	R-	KHKILAALLQDSSS	14.35	29.55	17.05	8.05	65.5	18.9	-3.35
TUK-1691-22	R-	KHKILHRAAQDSSS	13.95	7.15	14.55	12.1	1	14.6	7.6
TUK-1691-19	R-	KHKILHRAAQDSSS	5.75	7.1	6.25	-4.75	10.35	9.95	9.85
TUK-1661-47	R-	CFEQILHRLLGIDE	-18.3	2.45	5.35	-6.55	5.45	11.25	6.55
TUK-1663-25	R-	GPEQLLRHDIGIDE	-6.3	2.9	2.5	12.7	39.9	8.15	9.96
TUK-1663-29	R-	CPEQLLRKLICIDE	13.6	17.55	2.15	5.85	20.3	7.35	3.5
TUK-1665-41	R-	CFEQILTHLIGIDE	10.35	5.6	7.5	22.7	11.6	19.8	9.85
TUK-1665-47	R-	CFEQILTDLIGIDE	10.15	31.4	10.45	14.1	7.65	15.35	7.45
Perini									
TUK-1752-50	R-	LNKLLLDK	8.65	13.05	13.1	112.95	19.5	15.1	9.7
TUK-1752-61	R-	LVRKLLDK	2.5	2.35	11.5	4.65	7.5	9.55	2.7
TUK-1753-34	R-	LMRKLLDK	30.8	34.15	24.45	4.83	5.8	13.35	10.15
TUK-1753-37	R-	LMRKLLDK	0.7	31.5	13.1	10.85	13.85	13.75	13.6
TUK-1754-38	R-	LVRKYLDK	7.1	17.8	11.35	7.5	5.55	17.1	5.5

Peptide Activity in FP Assay

Receptors		RXR, 10ng/uL	LXRα, 25ng/uL	LXRβ, 20ng/uL	FXR, 25ng/uL	ERβ, 25ng/uL	PPARγ, 25ng/uL	PPARγ, 25ng/uL
Peptides \ Ligands		9cRA, 0.1uM	24,25 ex, 3uM	24,25 ex, 9uM	cDCA, 20uM	b-Estr., 0.1uM	BRL, 0.5uM	GW, 0.1uM
		ΔmP	ΔmP	ΔmP	ΔmP	ΔmP	ΔmP	ΔmP
TOK-1754-41	R- LVREYNGK	8.55	13.69	7.35	5.2	10.15	11.2	3.8
R-COR								
TOK-1676-40	R- ELEVLLQQ	8.05	9.65	9.7	0.35	8.3	19	11.05
TOK-1676-44	R- EDUNLLQQ	6.05	11.35	3.2	4.55	3.25	20.05	10.45
TOK-1785-49	R- ELITLADHIQIITQWAR	1.4	2.2	17.3	70.75	-6.25	6.0	7.7
TOK-1785-53	R- ELITLADHIQIITQWAR	30.45	11.25	10.65	77.65	0.45	10.85	4.05
TOK-1787-28	R- GSITQQTPA	20.65	11.85	8.5	11.15	8.85	10.5	1.25
TOK-1787-33	R- GSITQQTPA	19.6	13.85	7.2	11.75	14.05	0.6	13.35
TOK-1789-24	R- KGHVKSTEG	11.95	15.0	4.7	11.0	5.65	15.25	0.1
TOK-1789-28	R- KGHVKSTEG	7.0	9.4	4.29	9.55	9.6	0.1	14.45
TOK-1791-50	R- RTVTLAQRISEVITQPHTH	13.15	-0.9	3.65	1.65	6.75	14.05	14.05
[illegible]								
TOK-1671-16	R- KLLQLLTC	27.55	38.6	5.55	11.55	145.1	37.35	39.95
TOK-1671-26	R- KLLQLLTC	25.25	30.3	17.35	21.6	148.6	23.3	19.5
TOK-1673-34	R- ILHKLLQH	110.3	137.6	45.1	-6.6	92.7	48.45	45.2
TOK-1672-37	R- ILHKLLQE	42.15	25.15	10.3	7.75	33.3	13	16.85
TOK-1673-40	R- LLRYLLDR	95.65	40.75	-0.15	31.5	47.2	26.2	33.25